Investigation of the 2001-2003 IHN epizootic in farmed Atlantic salmon in British Columbia

Prepared by
Sonja Saksida BSc DVM MSc
Sea to Sky Veterinary Service
Campbell River, British Columbia

Table of Contents

Acknowledgements 2
Introduction 3
IHN 2001-2003 5
What is the case definition for IHN in Atlantic salmon? 5
What was the scope (i.e. how many farms were affected) of the recent epizootic? 5
What was the initial source of the infection? 5
What does the mortality curve associated with an IHN case look like? 6
What were the cumulative mortality rates associated with the cases of IHN? 6
Summary of IHN cases during the 2001-2003 outbreak 7
IHN cases in Area 1: 7
IHN cases in Area 2: 8
IHN cases in Area 3: 9
IHN cases in Area 4: 9
IHN cases in Area 5: 10
What changes were made at the sites after an IHN diagnosis was made? 11
What is the status of IHN as of December 2003? 11
Risk Factors and their management 12
Source of Infection 12
Freshwater introduction or vertical transmission 12
Horizontal transmission in seawater 13
A/ Wild sockeye salmon 13
B/ Waterborne transmission 15
C/ Other fish species that can carry IHNV 17
D/ Movement of farmed salmon in and between areas 19
E/ Shared equipment and common boat traffic 20
F/ Harvest by-products from IHNV infected populations 22
Other Risk Factors 24
Lag time between suspicion and confirmation of an infection 24
Stress in the Atlantic salmon 25
Lack of an effective IHNV vaccine 26
Research Needs 27
Summary 27
Figures and Tables 29
References 36

Acknowledgements

I would like to thank the British Columbia Ministry of Agriculture, Fisheries and Food (BCMAFF) and the British Columbia Salmon Farmers Association (BCSFA) for funding the study. I would also like to thank all the companies (Atlantic salmon producers and suppliers) who participated in the study. They were all extremely cooperative and very willing to spend a considerable amount of time helping me collect and validate the information presented in this document.

I would like to thank the Department of Fisheries and Oceans Canada (DFO), the Pacific Salmon Commission, Environment Canada and the District of Campbell River for being so prompt in providing me with information.

I would finally and especially like to thank Paula Galloway, Darlene Hanson, Gary Robinson, Garth Traxler and Dr. Craig Stephen for their assistance in editing the document. Their input was invaluable and, I know, increased the quality of the final product.

Introduction

Infectious hematopoietic necrosis virus (IHNV) is a serious enzootic pathogen that infects several species of salmonids in western North America and is the causative agent for the disease, infectious hematopoietic necrosis (IHN) (Traxler et al. 1998). Clinical signs of IHN include severe petechial and ecchymotic hemorrhaging in the viscera, as well as bloody ascites, and pale livers and gills (Traxler et al. 1998). IHNV is easily inactivated at temperatures of 32°C or greater and therefore does not pose a health risk to warm-blooded animals (birds and mammals) (Torgersen and Hastein 1995).

Horizontal transmission of the IHN virus occurs readily in both saltwater and freshwater (Traxler et al. 1998). Although there is anecdotal information that vertical transmission of IHNV occurs, no laboratory study has been able to demonstrate the event (Traxler et al. 1997, Bootland and Leong 1999). Ribonuclease protection assays and nucleotide sequencing of IHNV isolates collected over a 20 year period found that the isolates can be grouped into three separate regional isotypes: northwest coast (Oregon to Alaska), California, and Idaho (Hsu et al. 1986, Emmenegger et al. 2000, Troyer et al. 2000, Emmenegger and Kurath 2002). Different salmonid species appear to be sensitive to different genetic isotypes. For example sockeye salmon, *Oncorhynchus nerka*, are susceptible to the northwest coast IHNV isolates while chinook salmon, *Oncorhynchus tshawytscha*, are more susceptible to the Californian isolates. Rainbow trout, *Oncorhynchus mykiss*, are susceptible to the Idaho isolate.

Most of the losses associated with IHN, in sockeye salmon, occur in the freshwater alevin and swim-up stages. Disease in the seawater sockeye stages has not been as commonly observed, however, the northwest isolates are quite homogeneous indicating that continual mixing of the isolates has occurred over the generations. The only location where this appears possible would be in the ocean (Williams and Amend 1976, Traxler and Rankin 1989, Emmenegger et al. 2000). One such place could be the "Alaskan gyre", the common feeding grounds for all sockeye populations north of Oregon (Emmenegger et al. 2000, Emmenegger and Kurath 2002).

Lab transmission studies have found that Atlantic salmon are very susceptible to IHNV infections (Traxler et al 1993). In 1992, IHN was diagnosed in a population of farmed Atlantic salmon, *Salmo salar*, in British Columbia (BC). Over the next four years,

the disease spread to 13 separate farm sites within a 20 km radius of the index case (St-Hilaire 2000, St-Hilaire et al.2001). In the summer of 2001, nine years after the first epizootic, IHN was again diagnosed in a population of farmed Atlantic salmon. As in the 1992 epizootic, mortality as a result of the disease has been significant.

The British Columbia Ministry of Agriculture, Fisheries and Farms (BCMAFF) and the British Columbia Salmon Farmers Association (BCSFA) commissioned the following report with the objective to: describe the 2001-2003 epizootic, outline possible risk factors and to provide suggestions on how to minimize risks (including research needs). All companies with affected sites were interviewed. These companies were asked questions regarding their husbandry and management practices as well as specific fish history questions for each affected site. Similar questions, although not as detailed, were also asked about sites that remained IHN negative during the epizootic. Companies that did not have any IHN infected sites were not interviewed. The risk factors discussed in the report were based on questionnaire findings as well as the current knowledge of IHN in BC.

IHN 2001-2003

What is the case definition for IHN in Atlantic salmon?

For this report, an "IHN case" is defined as a farm site with Atlantic salmon confirmed positive for IHN. The date of IHN suspicion, based on clinical signs, was used to define the date a site contracted IHN. The date of confirmation of the virus by a diagnostic laboratory was not used because of the variable lag time (5 to 21 days) seen between suspicion and confirmation. For ease of understanding, case 1 occurred on the first site infected, case 2 on the second site infected and so on.

What was the scope (i.e. how many farms were affected) of the recent epizootic?

Between the August 2001 and June 2003, 36 Atlantic salmon farms developed IHN. Infections were identified in five separate areas; with the largest number of cases occurring in areas 1 (13 cases) and 5 (10 cases) (figure 1). All 14 Atlantic salmon farms diagnosed with IHN during the 1992 –1996 epizootic were located in area 1.

The first case, the index case, was diagnosed in late August 2001. The index case in the 1992 outbreak occurred in the same area and during the same time of year (summer) as this recent outbreak, perhaps indicating that the source of the viral exposure may be similar for both outbreaks.

What was the initial source of the infection?

Kurath (unpublished data) carried out ribonuclease protection assay (RPA) analysis and sequencing on 20 isolates in total collected from the 2001-2003 IHN outbreak. The assay determined that the 11 isolates examined from areas 1 to 4 were identical and very similar to the isolates that are widespread in Alaska, BC and Washington state. This isolate will be referred to as sequence A. The nine isolates

examined from area 5 were different from sequence A but still similar to the typical BC isolates (within the 2% nucleotide diversity range). This isolate will be referred to as sequence B. Kurath compared the isolates of the 2001-2003 epizootic (sequence A and B) with those of the 1992-1996 epizootic and found that they are different. This data suggests that the two isolates from the 2001-2003 outbreaks represent two independent new introductions of IHNV into farmed Atlantic salmon with both local sources being enzootic to BC: one source originating on the east coast and one on the west coast of Vancouver Island (Kurath unpublished data).

What does the mortality curve associated with an IHN case look like?

Detailed mortality data was examined from 20 infected sites. The shortest time from apparent exposure to the virus to clinical disease appears to be seven days. This was observed in a group of smolts shortly after being entered into seawater. Figure 2 shows a representative mortality curve associated with an on-farm IHN outbreak. The curve shows a steep increase in mortality shortly after diagnosis of IHN on the site, with mortality rates spiking six to eight weeks into the epizootic. The epizootic on the farm appears to last 20 to 22 weeks. A study examining the 1992 -1996 IHN epizootic found a second mortality spike 30 weeks after the first (St-Hilaire 2000), this was not seen in any of the cases in this recent epizootic.

The shape of the mortality curve indicates most likely a common point source for the infection (i.e. high number of viral particles) (Baldock 2000). The rapid increase in the mortality as seen by the steepness of the curve illustrates the highly contagious nature of IHNV in an Atlantic salmon population.

What were the cumulative mortality rates associated with the cases of IHN?

There is a statistically significant negative correlation between the size of salmon at onset of infection and cumulative mortality: with mortality rates highest in Atlantic salmon weighing less than 1kg, averaging greater than 70%. Mortality rates in salmon between 1 to 2kg and over 2kg averaged over 50% and over 40% respectively. These mortality rates appear to be higher than those found in the previous IHN epizootic (St-

Hilaire 2000). The cumulative mortality rates for cases where the farm populations were maintained on site for a minimum of 14 of the 22-week epizootic period are listed in table 1.

Summary of IHN cases during the 2001-2003 outbreak

Figure 3 illustrates the spread of the disease in the farmed Atlantic salmon population. Table 2 lists all the cases and their locations relative to the next closest positive site.

IHN cases in Area 1:

The locations of 13 IHNV positive sites in Area 1 are illustrated in Figure 4. The first three outbreaks were identified within twelve days of one another, from late August through early September 2001. These sites were located within 5 km of each other in the same channel. The fourth outbreak (case 4) was diagnosed one month after the third, and the fifth outbreak (case 5) an additional month later. By the end of November 2001, all five sites located within a 5 km radius had IHN. Harvesting commenced in December 2001.

The next two outbreaks in Area 1 (cases 9 and 10) were situated 30 km upstream from the original five cases. Cases 9 and 10 were located within 1 km of each other, on the same side of an inlet. IHN was detected in these cases in early February 2002, approximately 11 weeks after case 5. Less than one week later, the eighth site in Area 1 (case 11) was diagnosed positive for IHNV. This site was being harvested when the viral presence was confirmed. In the month prior to detection, chinook salmon smolts had been introduced onto this site.

Five weeks later the IHN virus was detected on the ninth site in Area 1 (case 14). This site was located 2 km across the inlet from cases 9 and10. At this point, there were still three IHN negative sites located between the sites with cases 9 and 11. These three sites, however, were diagnosed positive over the next month (cases 15,17 and 18). Two of the three sites, cases 17 and 18, were harvested out prior to laboratory confirmation of the viral infection.

Case 16 was diagnosed in March 2002. Some fish had been moved to this site in late January and early February 2002. These fish were towed in transport pens that passed in close proximity to a site which was diagnosed with IHN in early February 2002 (case 9). The site where the fish originated from was diagnosed with IHN in mid-March 2002 (case 14).

IHN cases in Area 2:

The first outbreak of IHN outside of Area 1 (case 6) was located over 100km north (figure 1). In this report, this area is referred to as Area 2. Figure 5 outlines the locations of the six IHN-positive sites in Area 2. Case 6 was a smolt site; IHN was suspected in mid-January 2002. Smolts had been delivered to the site via well boat between October 2001 and January 2002. The common path taken was through Johnstone Strait, through Area 1. As a precaution, the well boats transporting the smolts stopped pumping water into the holds containing the fish 40 km south of the IHN virus infected sites and resumed pumping 30 km north of the infected sites. The site was culled within three weeks of confirmation of the IHNV infection.

The second outbreak of IHN in Area 2 was case 12, located 3 km from case 6. Groups of fish that were being moved by towing transport pens to a new site, passed in close proximity to the site which would shortly become case 6. The site that received the transferred fish (case 12) was diagnosed with IHN seven weeks later.

The third outbreak in Area 2, case 21, appeared over five months (24 weeks) after the last outbreak in the area. This site was located 30 km from case 12, in a different channel. The fish on this site were entered in March 2002 and diagnosed with IHN August 2002. The fish were culled within three weeks after confirmation of the viral infection.

It was another five months before the fourth outbreak appeared in Area 2. That site, case 29, was diagnosed with IHN in late January 2003 while the site was being harvested. At the time the site was diagnosed, all sites containing previous IHN cases in Area 2 had been depopulated and the closest site still containing populations having undergone an IHN epizootic was in Area 3, located well over 100 km from case 29.

The fifth outbreak, case 34, was diagnosed 10 weeks after case 29. This site was depopulated within 1 week of laboratory confirmation of IHNV.

The final site diagnosed with IHN in Area 2 was case 36. Similarly to case 21, there were no other outbreaks of IHN in the area at the time of detection of case 36. The closest site having undergone an outbreak (almost one year earlier) was located 40 km away in Area 1. This site (case 36) was harvested out within a month of diagnostic confirmation.

IHN cases in Area 3:

Area 3 is several hundred kilometres north of Areas 1 and 2. There was only one outbreak of IHN diagnosed in this area, case 7 (figure 6). IHN was diagnosed at this site in mid-January 2002. This site contained smolts which had been transported via well boat, up Johnstone Strait through Area 1, between mid-October and the end of December 2001. As a precaution, the well boats stopped pumping seawater into the holds containing the salmon smolts approximately 40 km south of Area 1 and resumed pumping approximately 30 km north of the infected sites. In case 7, one pen was culled within 2 weeks of the initial diagnosis. The remainder of the site was culled approximately 16 weeks later.

IHN cases in Area 4:

There were six outbreaks of IHN diagnosed in Area 4 (figure 7), which is at least 80 km from either Area 2 or 3. The first site to contract IHN, case 8, contained Atlantic salmon that had been on the site for almost one year before becoming infected in early February 2002. The IHN virus was detected in this group approximately two weeks after case 7 (in Area 3). In July 2002, the survivors were relocated to another site in the area.

The second outbreak in the area (case 20) occurred in a population of Atlantic salmon smolts entered in March/April 2002. Case 20 was diagnosed in July 2002, five months (25 weeks) after case 8. The site containing case 20 was located 8 km downstream from case 8, however at the time of diagnosis, the population was being moved via well boat to the site where case 8 had originally been located. The site containing case 20 was culled by October 2003.

It was approximately another five months (25 weeks) before the next two outbreaks of IHN were diagnosed in Area 4 (cases 26 and 27), in January 2003. At the

time of diagnosis, the site (case 26) was being split and groups of fish were being moved across the channel, via well boat, to another site (case 27). Case 26 was located approximately 8 km downstream from the site that now held case 8.

The final 2 outbreaks, cases 32 and 33, were diagnosed with IHN 11 to 12 weeks after cases 26 and 27. These sites were located downstream 20km and 8km respectively, from case 27.

IHN cases in Area 5:

There were ten outbreaks of IHN diagnosed in area 5 located on the west coast of Vancouver Island (figure 8). The IHNV isolated from outbreaks in area 5 was characterized as sequence B, different from isolate found in the other four areas. The first site identified with IHN was case 13, a smolt site. Smolt entry was on-going when the IHN virus was detected, at which point smolt transport was stopped (mid-March 2002). The site was culled three weeks later. In mid-May 2002, case 19 was detected in area 5. This occurred 9 weeks after all fish in case 13 had been culled. This site contained salmon that had been in seawater for over one year. It was located 5km upstream from case 13.

It was approximately 25 weeks (October 2002) before the next outbreak (case 22) of IHN appeared in Area 5. This site was located 7km downstream from case 19. Nine to ten weeks later, in December 2002, three new outbreaks appeared—cases 23-25. Two of the outbreaks, cases 24 and 25, were located upstream 5 and 12km respectively, from case 19. Case 23 however, was located in an entirely different channel over 30 km away from all previous cases diagnosed in the area. Within four weeks, three more sites were diagnosed with IHN: case 28 located 3 km across and slightly downstream from case 23, case 30 a smolt site 18km upstream from case 23, and case 31 located 6km upstream from case 28. In summary, within a one-month period, from the end of December 2002 to the end of January 2003, a surprising six new cases of IHN were diagnosed in area 5.

The final site in area 5 to be diagnosed with IHN was case 35—a smolt site—diagnosed with IHN in April 2003, 11 weeks after the previous case.

What changes were made at the sites after an IHN diagnosis was made?

All sites established isolation protocols when a diagnosis of IHN was made. The rate of mortality removal was increased; in many cases during the initial high mortality periods dives occurred daily. Secondary non-diving recovery systems, such as "uplift" and "mort ring" systems, were also set up on several farms.

Harvesting of confirmed positive populations were conducted according to the Department of Fisheries and Oceans Canada (DFO) protocols with the exception of two cases where harvesting was completed (case 17, 18) before laboratory confirmation of IHNV was received.

Even though IHN is not a reportable disease, all companies kept the provincial and federal government agencies informed of positive results.

What is the status of IHN as of December 2003?

During the period of IHN epizootics in the area, three sites remained negative in area 1. These sites were located in a separate channel and contained Atlantic salmon that had been entered into seawater in the spring of 2002. As of September 2003, there were no longer any farm sites with Atlantic salmon that had survived an IHN outbreak remaining in area 1 (figure 4).

In area 2, all sites having undergone an IHN outbreak were depopulated by August 2003. During the epizootic period, there were several Atlantic salmon sites within a 10 km radius of the infected sites that never contracted IHN (figure 5).

In area 3, the one positive site was culled. The remaining two sites situated 20km from the infected site remained IHN free (figure 6).

In area 4, all the Atlantic salmon farms in the area eventually contracted IHN (figure 7). This area is to be depopulated by early 2004.

In area 5, all but one Atlantic salmon farm eventually contracted IHN (figure 8). All IHN positive sites were depopulated by the end of 2003.

Risk Factors and their management

The following section outlines possible factors associated with increased risk of IHN in Atlantic salmon. In addition, suggestions for minimizing risks beyond the BCSFA code of practices are presented[1].

Source of Infection

Freshwater introduction or vertical transmission

It is known that horizontal transmission of the IHN virus can occur in fresh water and there is evidence of vertical transmission, although it has not been demonstrated in controlled laboratory conditions (Bootland and Leong 1999). There is, however, no evidence to indicate that IHNV infections in the cases discussed in this report, as well as those that occurred during the 1992-1996 epizootic (St-Hilaire 2000), were introduced from freshwater sources. In the present epizootic, there were fifteen hatcheries that supplied smolts to both infected and non-infected sites. Four of the 5 companies routinely screened Atlantic salmon brood stock for IHNV at egg-takes. There have never been any positive results.

In addition, the Atlantic salmon involved in the first three cases had been in seawater for over one year before showing signs of IHN. Research has found that Atlantic salmon are very susceptible to IHNV infections and become clinically ill shortly after exposure (Traxler et al. 1993). There was no evidence to indicate that there had been any unusual mortality in these groups in fresh water. Finally, there was no Atlantic smolt movement in the area preceding the index case (case 1).

How can this risk be minimized?

- Avoid using brood fish from areas where IHN has been diagnosed.

- Continue to screen all brood stock for IHNV. Current methods include viral culture and/or serology, however, better diagnostic methods may be developed in the future. Immediately destroy any populations diagnosed positive with IHNV.

[1] Developed in conjunction with BCSFA

- Ensure stringent disinfection of utensils, facilities, external surfaces of brood stock etc., during and after egg collection.

- Presently, most producers also treat eggs with iodophor—100 parts per million (ppm) for 10 minutes—prior to incubation. In Alaska, sockeye eggs are required to be water hardened in 100ppm iodophor for 60 minutes to reduce the potential of IHN virus contamination (McDaniel et al. 1994). Taking into account the egg size and fecundity differences between the species, a similar protocol should be developed for Atlantic salmon eggs.

- Use an IHNV-free water supply at the hatchery.

- Conduct viral screening prior to moving fish between areas (i.e. hatchery to seawater sites). A suggested sampling procedure is outlined in the DFO Fish Health Protection Regulations: Manual of Compliance (1984).

Horizontal transmission in seawater

A/ Wild sockeye salmon

There is some evidence that wild salmon could have been the source of the infection for the initial few cases. For example, the first three sites to be diagnosed with IHN were all located in the same channel. Infection was detected in late August 2002, at which time there were a large number of returning sockeye, chinook and coho salmon in the channel. IHN is a common disease of sockeye salmon known to cause high mortality in early life stages (Williams and Amend 1976, Traxler and Rankin 1989). Ribonuclease protection assay (RPA) work found that the IHN virus isolates from the farmed salmon are similar to those found in local sockeye salmon (Emmenegger et al. 2000, Emmenegger and Kurath 2002, Kurath unpublished data).

Normally, sockeye return from the common feeding grounds off Alaska to freshwater as four or five year olds (Emmenegger et al. 2000, Groot 1996). The sockeye populations that would potentially pass through Area 1 to spawn would include many of the Fraser River runs, as well as many minor runs that enter the local rivers and

lakes, such as the Philips Arm, Bute Inlet and Heyden River runs. Figure 9 shows the sizes of the Fraser River runs from 1990 to 2001 (from the Pacific Salmon Commission). In 1992 and 2001, the total production for the Fraser River runs was 6.4 and 7.2 million respectively. This is considered a moderately low return. Low runs can be cyclical, or may be related to some external environmental factors such as water temperature, lack of food or even disease. There is very little data available on the non-Fraser stocks.

We do not know what factors led to the 1992 and 2001 IHN epizootics in farmed Atlantic salmon. One hypothesis is that a certain group of returning wild salmon had higher IHNV prevalences and titres than usual and, consequently, were shedding more virus particles. Another hypothesis is that the first group of Atlantic salmon to become infected were stressed and therefore more susceptible to the infection. A third hypothesis is that even small variations in genetic sequences of the virus affect the virulence of the virus in Atlantic salmon and there is one or more stock of wild salmon, which are carriers for this more virulent variants. As a result of the cyclical nature of the sockeye life history, this variant would only appear every four to five years with the return of this stock.

A large study conducted by Meyers et al (2003), examined IHNV data collected by the Alaskan sockeye culture program between 1981 and 2000. They found that the prevalence of IHNV in the adult sockeye salmon fluctuates from year to year. Generally, the prevalence of infected salmon and the percentage of those infected with high IHNV titres peak and decline together. Interestingly, in 1992, the same year the first IHNV epizootic started in farmed Atlantic salmon in BC, the IHNV prevalence and the percentage of spawning Alaskan sockeye salmon with high viral titres were both some of the highest recorded levels–56.2% and 65.7% respectively. Unfortunately there has been no comparable work done on adult sockeye salmon returning to British Columbia rivers.

Research has shown that Atlantic salmon can become infected when placed in close proximity to an infected salmon (Traxler et al 1993). However, the minimal dose and exposure length required for transmission is not known.

How can this risk be minimized?

At this time, knowledge is insufficient to be able to establish protocols that would, in effect, eliminate this risk. Therefore, research in this area is critical. There should be a concentrated effort to support disease research in wild fish.

B/ Waterborne transmission

Survey results indicate that once an Atlantic salmon pen becomes infected with IHNV and breaks with the disease, the disease quickly spreads to the other pens on the site. The probable mode of transmission is via waterborne exposure. The IHN virus can survive in salt water. Studies have shown that 99.9% of IHN virus is inactivated after 2 weeks in saltwater at 15°C (Toranozo and Hetrick 1982). However, given high enough titres in the environment, the 0.1% remaining can still represent a significant source of infection. There are indications that IHNV survival may be even longer at lower water temperatures (Torgersen and Hastein 1995). The water temperature during the present epizootic ranged from 7 to 11°C.

On the British Columbia coast, because of the abundant rainfall and consequently the large amount of freshwater run-off, there is a net surface outflow of tidal water from the inlets and channels. This water movement is therefore more analogous to a river rather than to a simple back and forth tidal action. Figure 10 illustrates this predominate uni-directional flow of surface water in Area 1. Similar outflow patterns appear to occur in the other four areas. It is possible that if there is a high enough IHN viral titre in the water, it could spread to sites located "downstream" from diseased sites. Overall, the titres associated with waterborne exposure would be expected to be lower than direct contact with an infected fish, and as a result, the time of onset from exposure to clinical disease may be prolonged.

If the assumption is made that the east coast Vancouver Island isolate originated in Area 1, then waterborne exposure of the smolts, in Areas 2 and 3, to IHNV could have occurred as they were being live hauled through Area 1. In addition, a low titre exposure to the virus may explain why the onset of disease in the population did not appear until several weeks after the last group of smolts had been entered.

The presence of an IHN positive population, undergoing an epizootic, could provide a continuous reservoir of virus not only for the infected site itself but also to the

surrounding area (i.e. downstream). All of this is merely a hypothesis, however, and further research is required.

How can this risk be minimized?

- Establish management zones. The zones would be established on the basis of oceanographic and geographic confines (i.e. areas with shared water movement, land masses). All farmed fish activities should be maintained within the zone, including harvesting. Movement between zones should be limited. Defining a "zone" will be difficult considering the number of variables and lack of knowledge.

- Upon obtaining a positive IHN diagnosis, all other companies operating in the management zone, as well as relevant contractors should be informed. Movement of fish between management zones should stop. All companies operating in other management areas should also be informed.

- A security ring should be placed around infected sites. This area is then considered a high-risk and cross-area movement should be restricted. An arbitrary security ring size of three km or one tidal "flush" (whichever is larger) could be used until a more realistic size is determined. A more appropriate security ring size should be established for each site based on local oceanography and could be adjusted, as more information on survival and dispersion of IHNV in seawater becomes known.

- Depopulation of the infected site should occur as quickly as possible.

- Processing of infected fish should occur exclusively at processing plants in the same zone or at plants with "complete" water disinfection.

- All "uninfected" sites in the same management zone as the infected site should increase virus/disease surveillance. Viral testing of "suspect" mortalities should be conducted at all 'uninfected' sites immediately upon notification of a positive IHN diagnosis. Monthly testing of "suspect" mortalities should continue until at least two to three months after all infected sites have been depopulated.

- Do not introduce Atlantic salmon to a zone with IHN positive sites.

- Do not use transport pens to move fish within or between infected zones.

- Do not transport live fish through infected areas while circulating seawater through their holding tanks.

- After all sites are depopulated, fallow the infected zones for a period of time, agreed upon by all the stakeholders, in consultation with appropriate technical experts.

- In an attempt to reduce waterborne transmission, a "no pumping" zone should be placed around all processing plants (wild and farmed fish). The size of the zone should be area specific and based on local oceanography. When within the zones, well boats hauling live fish would be expected to cease pumping water into the holds containing fish. Similar to that suggested for infected sites, an interim zone size could be three km or one tidal flush (whichever is more) from all processing plants (wild and farmed fish). The size of the zones may need to be adjusted with increased knowledge of IHNV.

C/ Other fish species that can carry IHNV

Disease can be spread by carriers; animals that are infected and capable of transmitting the infection but are not themselves diseased (Baldock 2000). IHNV carrier fish species that are migratory would be a very effective vector for the virus and could provide a possible explanation as to the spread of IHN to sites located "upstream" from infected sites.

During the 2001-2003 IHN epizootic, there were several cases where migratory fish populations were seen in the vicinity of the sites, prior to IHN being detected. For example, the index case was detected during the summer of 2001. At that time, large numbers of adult sockeye, chinook, and coho salmon were assembling in the area on their regular migration back to the rivers to spawn. In coastal BC, IHN is considered a

disease of sockeye salmon, but not of chinook or coho salmon. St-Hilaire et al. (2001), however, presented evidence showing that chinook salmon could carry the BC variant of IHN, without exhibiting signs of disease, yet could still infect Atlantic salmon. Carrier states in chinook salmon should, therefore, not be ruled out as a possible source of infection in the case where IHN was detected shortly after chinook smolts were entered onto a site still containing Atlantic salmon.

Species other than salmonids may also act as carriers for IHNV. In 12 of the IHN cases, diagnosed in the early parts of the year in 2002 and 2003, large populations of Pacific herring (*Clupea harengus*) and pilchard (*Sardonops sagax*) were reported in the vicinity of the sites prior to their outbreak. One of these cases was case 13; the first of the sequence B variant cases in area 5. Although herring appear quite resistant to IHNV, laboratory challenge studies have shown that herring can harbour low viral titres in their tissue, indicating that they could serve as carriers of IHNV in nature (Kocan et al. 1997, Traxler et al. 1998). Additionally, in a wild fish disease survey conducted by DFO, IHNV was isolated from a Pacific herring collected from Departure Bay, Vancouver Island; an area not associated with salmon farms (Kent et al. 1998).

It is also quite common to see small marine species such shiner perch (*Cymatogaster aggregata*) and tubesnout (*Aulorhynchus flavidus*) in and around the pen systems. Traxler and Richard (1996) isolated high titres of IHNV from apparently healthy shiner perch and tubesnout collected at a fish farm undergoing an IHN epizootic, indicating that they could act as vectors or reservoirs for IHNV. Unfortunately, there is nothing known of the home range of these marine species; for example, do they remain resident at a site or do they travel between sites.

How can this risk be minimized?

- Avoid concurrently raising Atlantic and chinook salmon (or other possible IHNV carriers species) on the same sea site.

D/ Movement of farmed salmon in and between areas

The risk of spreading IHN by moving Atlantic salmon within an infected area is very high. The level of risk appears to be strongly associated with the mode of transport used for moving the fish. For instance, the towing of pens with fish from one site to another appears to pose a very high risk of spreading IHN. There appear to have been several cases that developed as a result of exposure to infected (but undiagnosed) pens of Atlantic salmon that were towed past uninfected sites during their move to a new site. And conversely, pens of uninfected Atlantic salmon exposed to IHNV as they travelled past diseased sites while being moved to other sites.

The use of converted fish boats/packers, rather than well boats designed specifically to haul live fish, for moving live salmon to sites may also pose a higher risk of spreading IHN. This is because the deck hatches or water inlets of the holds of these vessels cannot be completely sealed. Consequently, there may be a small risk of infection associated with the use of these vessels even when their seawater circulation systems have been closed.

There also appear to be cases where infected, but undiagnosed, Atlantic salmon were transported via live-haul boats to a new site and subsequently infected farms situated downstream from the new site. In one case, Atlantic salmon may have become infected as a result of contact with exposed farmed chinook smolts introduced onto the site.

How can this risk be minimized?

- Transport salmon using suitable vessels (well boats), rather than towing cages with fish.

- Avoid movement of fish within IHN confirmed areas.

- Avoid multi-year class sites

- Avoid pumping water into well boats three km or one tidal flush (whichever is more) from all processing plants (wild and farmed fish) and all infected sites. (See *waterborne transmission section for rational*).

- Conduct routine viral testing on a regular basis.

E/ Shared equipment and common boat traffic

The risk associated with equipment and vessel movement is exacerbated by failure to remove/disinfect infected organic material or water, thereby possibly increasing the risk of transferring the virus to new locations. Risk is particularly high with larger vessels that use water for ballast or cooling and for large pieces of equipment such as pen systems and buildings. However, the removal of all organic material capable of carrying the virus below the water line is very difficult.

Multi-site contract vessels can also pose a problem due to high frequency of inter-site travel. It is possible in one of the present cases that IHNV could have been transmitted in this manner. For example, in the case where chinook smolts were delivered to a site while Atlantic salmon were still being harvested, the contract vessel used for the transport had been the main vessel used to haul infected mortalities from several IHNV positive sites. This was unknown by the farming company entering the smolts. This site subsequently became infected.

Using the right protocols, the IHN virus can easily be killed by various chemical (i.e. iodophors, sodium hypochlorite) and physical disinfectants (i.e. heat, ultraviolet light).

How can this risk be minimized?

- Monitor and record high-risk boat movement (i.e. boats that travel to multiple farm sites) to/from a farm site. Restrict all unauthorized boat traffic within site lease areas.

- If IHNV infection is diagnosed on a site, the site should be isolated. All movement of equipment on the site and between sites should cease while management plans are re-assessed and biosecurity tightened.

- Establish good communication lines between individual fish farming companies and outside contractors (i.e. marine service providers) so that sites are notified of any suspected infections.

- Large vessels should not draw seawater into holding tanks, for ballast, for engine cooling or any other purposes while within the security ring established around infected (suspect and confirmed) sites or process plants processing infected salmon.

- Establish industry-wide minimal level disinfection protocols for farming companies and contractors.

- All well boats should be appropriately cleaned and disinfected between handling fish from different sites or fish groups.

- Use separate vessels for live hauling fish and IHN mortality removal from sites.

- Random audits of well boats should be conducted (disinfection records, specific testing of decks and hold surfaces)

- Where possible, equipment and personnel should be designated to individual sites. Where transfer between sites is unavoidable, the level of cleaning and disinfection effort would be based on the risk of transfer of the pathogen. Each piece of equipment or equipment class should be rated in relation to risk and efforts to disinfect should be applied accordingly. Examples of high-risk equipment include all nets, mortality rings and buckets. Examples of low-risk equipment include microscopes, computers and other equipment not in direct contact with the salmon, the seawater or net pen system.

F/ Harvest by-products from IHNV infected populations

Harvesting of IHNV positive fish started in December 2001. These populations were stunned and bled on site and then shipped and trucked to the processing plant for processing. All harvest and blood water was contained and treated as per protocols specified by DFO, along with BCMAFF, prior to discharge. During this time, two companies were transporting smolts through Area 1 to two separate sites. Both companies had protocols in place to cease pumping water into the holds from 40 km "upstream" from the channel containing sites with IHNV infections to 27 km "downstream" from the point where the harvested fish from infected sites were being offloaded onto trucks. Both companies however, were pumping water into the holds as they travelled past the fish plant that was processing salmon from the IHN positive sites. The fish plant was located just outside the water turn-off point. It is possible that even with the adherence to the prescribed disinfection protocols that some live virus may have been discharged by the processing plant and then subsequently pumped up into the well boats containing the smolts. If the well boats then stopped pumping a short distance past the process plant the smolts could possibly be exposed to IHNV contaminated water during the entire time that pumping of fresh seawater has stopped. Although this would seem to be an unlikely event, there was single day during which an infected site was being harvested and processed concurrently to smolts being transported through the area to two separate uninfected sites in Areas 2 and 3. Both these sites contracted IHNV shortly thereafter.

Others companies have shipped smolts via well boats through Area 1 since the above-mentioned event. However, the stop-pumping point had been re-adjusted to start 25 km south of the fish plant processing fish from IHN positive sites. After the change was implemented, no new IHN outbreaks occurred at newly entered smolt sites in Areas 1 through 4. In addition, by the end of 2003, most plants that process farmed salmon had set up treatment facilities to successfully disinfect harvest by-products.

The initial protocol deemed acceptable by DFO and BCMAFF for handling harvest blood water and processing water involved chlorination (with levels required to reach five ppm active chlorine for a minimum of 10 minutes) and dechlorination (using sodium thiosulphate to a reach a chlorine level of less than 0.01 ppm prior to discharge). With the exception of the two cases where confirmation of the virus was made after harvesting of the site had been completed, all sites harvesting infected fish adhered to

the protocol for blood water and processing water disinfection. Concern with the ability to fully contain the blood water during on-site stunning and bleeding resulted in certain companies altering their harvesting protocol, so that fish were only killed by stunning (by a percussive blow to the head) on site and shipped, unbled, to the processing plant.

There are some concerns regarding the effectiveness of the protocol, specifically regarding the presence of organics, pH and mixing capabilities. Most of the water being treated contained large amounts of organic material, and in the presence of organic material chlorine is known to be inactivated to varying degrees, depending on the concentration of organics (Torgersen and Hastein 1995). Interviewed company representatives who had used the prescribed protocol found that it was very difficult to reach and maintain the recommended level of chlorination. The higher pH levels of seawater—8 compared to 6.5 to 7.0 in freshwater—actually changes the active form of chlorine from hypochlorous acid to hypochlorite which is100x less effective as a disinfectant (Washburn et al. 1998). There is an added level of concern with the protocol, when it was noted by some during the interview, that the addition of salt water artificially increased the detectable level of chlorine. Finally, there was some difficulty ensuring complete mixing of the chlorine and wastewater. The question arises as to how effective the protocol is in destroying the virus in large volumes of water, especially when complete mixing is difficult and organic levels are high. It is possible that there may have been some unintentional releases of IHNV infected water.

As stated earlier, most plants that process farmed salmon in BC have set up treatment facilities to successfully disinfect harvest by-products. However it has to be remembered that IHN is also a common disease in wild salmonids in BC such as sockeye and the majority of the processing plants that handle wild fish still do not treat their process water prior to discharge. These facilities could pose a substantial risk to the farmed fish population.

How can this risk be minimized?

- Evaluate the effectiveness of the current processing plant waste discharge protocols mandated by the federal and provincial authorities for both wild and farmed fish operations.

- Government and industry should work together to develop new effective standards for handling process and blood water by both wild fish and farmed fish processing plants.

Other Risk Factors

Lag time between suspicion and confirmation of an infection

For sites where mortality data was examined, in 82% of the cases IHN was suspected prior to any substantial jump in mortality. This indicates a high level of diligence in fish health monitoring at the sites. The concern, however, is the length of time between suspicion and confirmation of IHN. This ranged from five to twenty-one days, with an average of 15.7 days.

Presently the "gold standard" for IHN confirmation is viral isolation via tissue culture methodology. Epithelioma papulosum cyprini (EPC) and chinook salmon embryo (CHSE) cells are the common cell lines used to isolate IHNV. Cell and tissue morphology changes known as cytopathic effects (CPE) are seen in cell lines infected with the IHN virus. The time it takes to see CPE may vary between labs due to variations in cell lines and lab procedures as well as shipping time, condition of the samples and the virus titre in the collected samples. The shortest time was approximately four days; however, other labs took as long as ten days to obtain CPE in their similar, but not identical, cell lines. Although a presumptive diagnosis may be made based on CPE of culture, a virus-specific test is required to confirm IHNV. The two tests used during the present outbreak were indirect fluorescent antibody tests (IFAT) and reverse transcriptase polymerase chain reaction (RT-PCR). These confirmatory tests may take an additional 24 to 48 hours.

Many of the management changes would only occur after laboratory confirmation of IHNV, since the associated costs would be significant. Consequently, delays in diagnosis increase the prospect of not tightening the biosecurity barriers quickly enough.

There is an RT- PCR test that can be used directly on fish tissue and could supply results in less than 2 days. This technique, however, has not been validated and thus cannot be considered the definitive diagnostic test for IHN. As well there is concern that this technique cannot distinguish fish that had been vaccinated against IHNV from

fish that are infected with the virus; possibly arising in false positive results. Presently, there is still a wide variation in lab results using this technique. Proper validation and standardization of this protocol could make it a valuable tool in the future.

Viral isolation appears to be most effective when a Atlantic salmon population is undergoing an outbreak; it is difficult to detect IHN virus in survivors with this method after the mortalities have stabilized (St-Hilaire et al. 2001). Serology has been found to be a more effective tool in determining whether the virus is still present in a population. However, the use of certain types of IHN vaccines could produce false positive results (St-Hilaire et al. 2001).

How can this risk be minimized?

- Validate the tissue PCR test.
- Standardize tests between laboratories.
- Develop other rapid diagnostic tests for detecting IHNV.
- Develop a test to screen and detect IHNV carriers.
- More precisely define clinical signs and proactively react with biosecurity measures at first suspicion of a viral agent.

Stress in the Atlantic salmon

The data appears to indicate that several of the populations were undergoing some elevated levels of stress prior to the onset of IHN. Unfortunately, the scope of the study did not allow for evaluation any strength of association for any of these stressors, and there are no "tools" for quantifying stress levels. It appears that the mortalities were highest in pens that were "stressed". However, populations that appeared not to be stressed also became infected. A stress could include: acute disease events other than IHN, a chronic disease condition such as bacterial kidney disease (BKD), vaccine adhesions, environmental (i.e. plankton, predation, low dissolved oxygen), and handling (i.e. grading, splitting, harvesting). All IHN positive sites used some combination of

predator nets, shark guards and treated nets, in addition, most sites with fish less than one kg had bird nets over the system. These barriers, although reducing stress associated with predator attacks, do not stop the movement of wild fish through the mesh of the pens (especially the smaller species of wild fish that could pose a risk to the farmed Atlantic salmon).

How can this risk be minimized?

- Avoid excessive handling of infected populations or populations near infected sites.

- Develop tools to monitor and reduce stress in the farmed salmon

Lack of an effective IHNV vaccine

All the Atlantic salmon smolts were vaccinated with commercially available bacterin vaccines. Though the majority were vaccinated in freshwater, on rare occasions, saltwater vaccination may have occurred. In six of the IHN cases, fish were vaccinated against IHNV using an autogenous vaccine, produced by a commercial vaccine supplier, at the specific request of an aquaculture company. Data collected from those cases where the fish were maintained in saltwater for the duration of the epizootic showed variable protection. In one case the cumulative mortality was 24%; substantially lower than the average mortality of 50% for fish of similar size range (table 1). In contrast, in two other cases no protection was observed; and the cumulative mortality observed (77 and 80% respectively) were equal or higher than the average mortality rate for fish of similar size (70%).

From a technical standpoint IHNV is an excellent candidate for a vaccine since it causes a rapidly spreading disease that involves multiple animal species (Atlantic and Pacific salmon species) (Kahn et al 2002). From an industry standpoint, IHNV is an excellent candidate for a vaccine since solving the problem is essential to the well being of the industry. However, at present there are no effective IHN viral vaccines commercially available.

How can this risk be minimized?

- Develop and implement industry-wide use of an effective licensed IHNV vaccine.

Research Needs

The general lack of knowledge regarding IHNV in wild and farmed fish populations makes it difficult to formulate practical, yet effective, risk management practices. Therefore, more research is necessary to further understand and control the disease. The following list includes some of the research needs that should be supported.

- Studies of IHN in wild fish species in order to determine:
    a. IHNV prevalence and titre level in wild sockeye populations,
    b. Differences in virulence of IHNV variants,
    c. Dose and exposure levels for infection,
    d. Identification of IHNV carriers in commercially and non-commercially important species,
    e. Modes of transmission of IHNV by wild fish species in the various zones.

- Continued monitoring of the IHN pathogenesis in the Atlantic salmon populations to include:
    a. Validation of the risk factors,
    b. Elucidation of the importance of the carrier state in transmission and spread of IHNV within Atlantic salmon populations,
    c. Government and industry should work together to develop new effective standards for handling process and blood water by both wild fish and farmed fish processing plants.
    d. Development of effective detection and mitigation management tools (i.e. vaccine, quick and effective diagnostic tests).

Summary

It has to be re-emphasized that IHNV is enzootic to the west coast of British Columbia and over time indigenous species of fish have adapted to the virus. Atlantic salmon, however, are a newly introduced farmed species and, therefore, are naïve to the infection. The disease process is very aggressive and acute in this species. This document outlined the 2001-2003 IHN epizootic in farmed Atlantic salmon from its onset in August 2001 to the end of December 2003. As of the time of the completion of the report, no sites were experiencing elevated mortalities associated with an IHN outbreak

and by March 2004 it is expected that all sites containing Atlantic salmon that had undergone an IHN outbreak will have been depopulated. In the report, potential risk factors associated with the disease were outlined and suggestions were made on how to minimize them, based on current knowledge. This should be regularly reviewed and modified with the advancement of understanding of the virus and controlling of the disease.

Figures and Tables

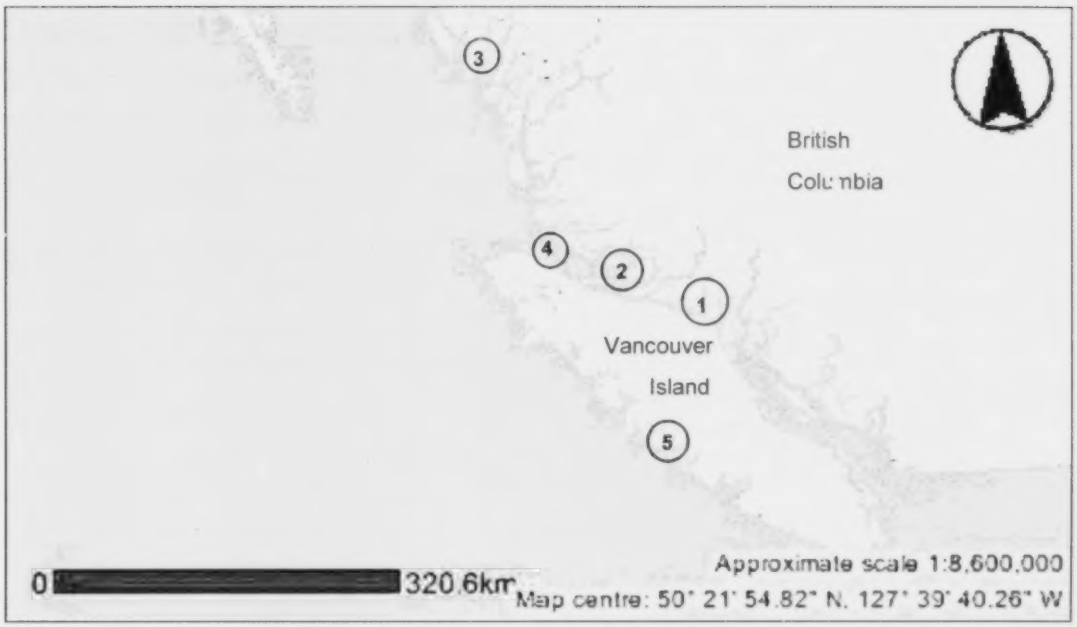
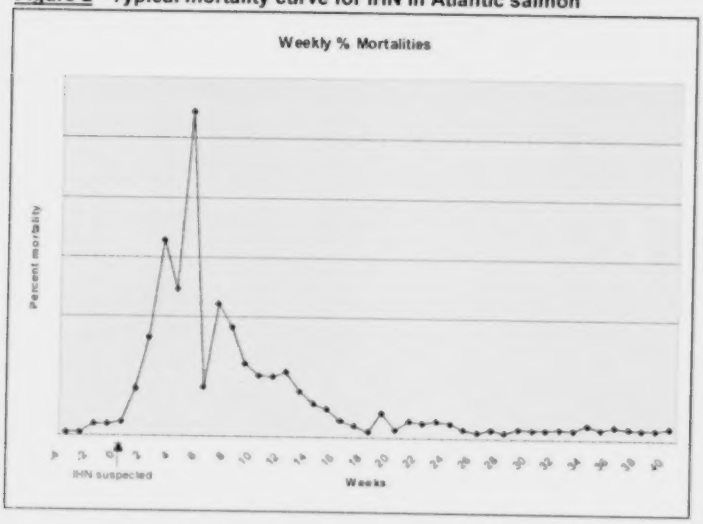


Figure 1 - Vancouver island showing the 5 areas with IHNV infections (source www.fishwizard.com)

Figure 2 - Typical mortality curve for IHN in Atlantic salmon



Cumulative mortality

cases where the exposed populations experienced a minimum of 14 weeks of the IHN epizootic (i.e no culling, no harvesting)

<1 kg	1- 2 kg	>3kg
94%	74%	58%
80%	73%	53%
79%	65%	47%
77%	62%	26%
71%	51%	20%
68%	46%	
62%	42%	
45%	24%	

Table 1 - Cumulative mortality rates in different sized Atlantic salmon during the IHN epizootic in 2001-2003

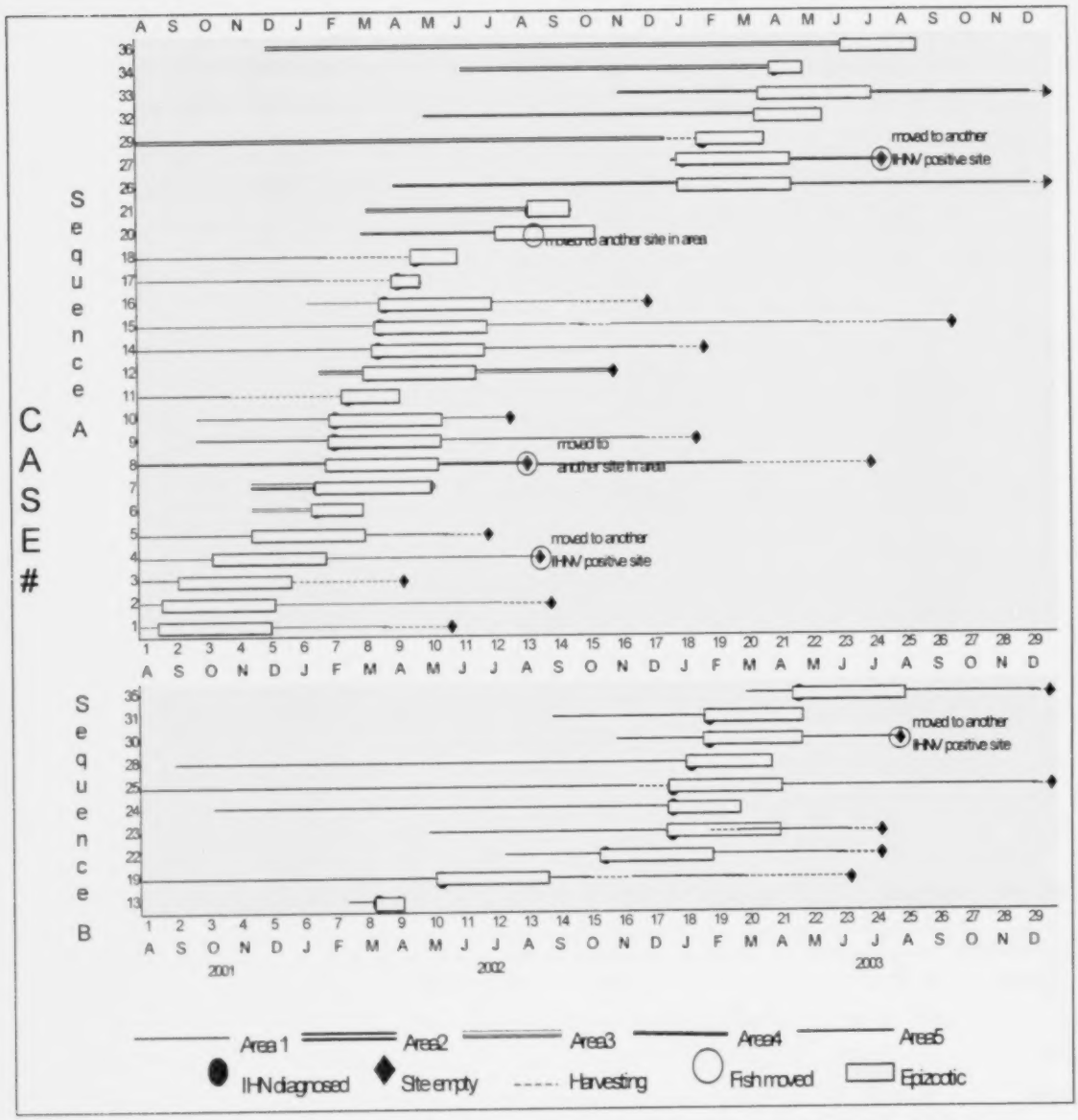


Figure 3 - Schematic diagram illustrating the progression of the IHN virus in affected Atlantic salmon fish farms in BC

Case	Area	Days from index case	Weeks from previous case in area[1]	Location relative the closest infected site	Distance from closest infected site (km)	Average weight of fish on site (g)
1	1	0				3000
2	1	4	<1	Downstream	3	2490
3	1	12	1	Downstream	5	5230
4	1	42	4	Cross/down	6	660
5	1	80	5	Cross stream	3	4580
6	2	135	0	Different area	>100	159
7	3	137	0	Different area	>100	176
8	4	150	0	Different area	60	1132
9	1	155	11	Upstream	30	500
10	1	156	<1	Upstream	1	96
11	1	161	<1	Downstream	10	5900
12	2	183	7	Different channel	3	1200
13	5	188	0	Different area	>100	60
14	1	196	5	Cross stream	2	687
15	1	197	<1	Cross stream	4	1800
16	1	203	<1	Different inlet	15	1800
17	1	210	1	Upstream	5	4630
18	1	225	2	Downstream	3	6800
19	5	253	9	Upstream	5	1900
20	4	326	25	Downstream	8	418
21	2	353	24	Different channel	30	290
22	5	425	25	Downstream	7	1590
23	5	486	9	Different channel	30	2150
24	5	489	<1	Upstream	5	4100
25	5	491	<1	Upstream	12	4900
26	4	502	25	Downstream	8	925
27	4	502	0	Cross stream	17	1170
28	5	506	2	Cross/down	3	3500
29	2	520	24	Different area	55	5000
30	5	521	2	Up stream	18	220
31	5	521	0	Up stream	6	3500
32	4	577	11	Downstream	20	4000
33	4	583	<1	Downstream	8	1800
34	2	590	10	Up stream	5	6500
35	5	597	11	Downstream	3	130
36	2	660	10	Different area	40	4500

[1] rounded to closest whole week

Table 2 – Lists IHNV positive sites, dates of detection relative to the index case and relative locations to the next closest positive site. Average weights of the fish at time of detection are listed also.

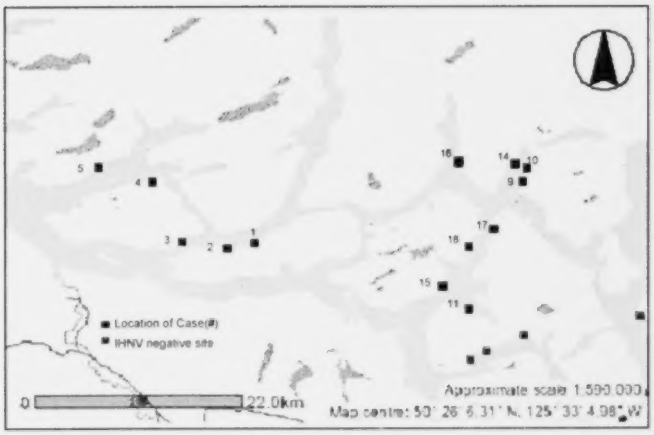


Figure 4 – Shows locations of IHN cases in Area 1 as well as closely situated uninfected sites.
(map source www.fishwizard.com)

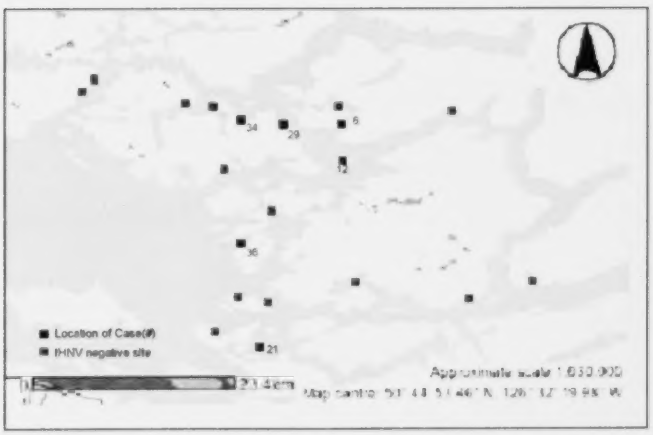


Figure 5 – Shows locations of IHN cases in Area 2 as well as closely situated uninfected sites.
(map source www.fishwizard.com)

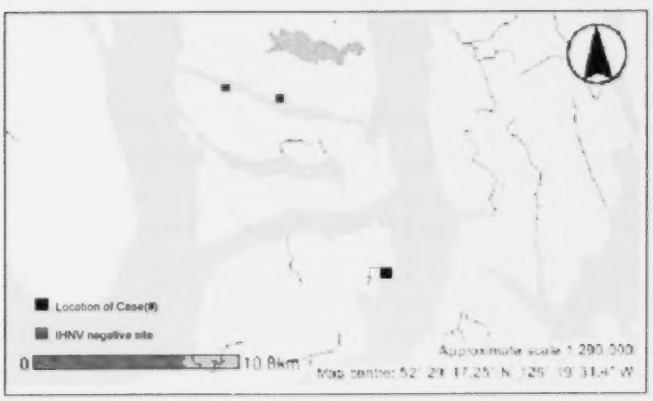


Figure 6 – Shows the location of the IHN case in Area 3 as well as closely situated uninfected sites.
(map source www.fishwizard.com)

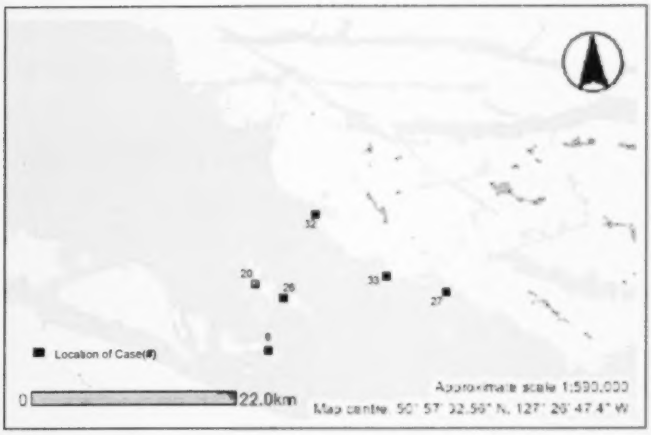


Figure 7 – Shows locations of IHN cases in Area 4.
(map source www.fishwizard.com)

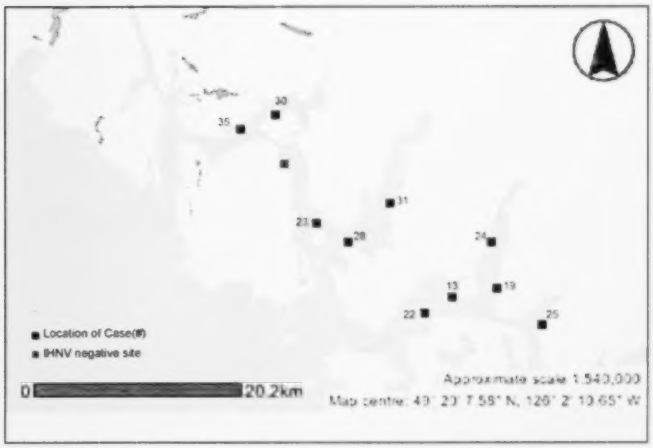


Figure 8 – Shows locations of IHN cases in Area 5 as well as closely situated uninfected sites.
(map source www.fishwizard.com)

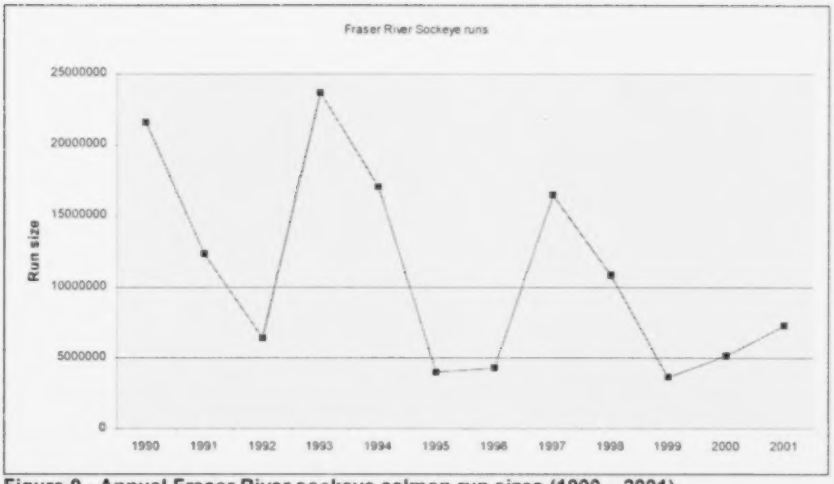


Figure 9 - Annual Fraser River sockeye salmon run sizes (1990 – 2001)
(source: Pacific Salmon Commission)

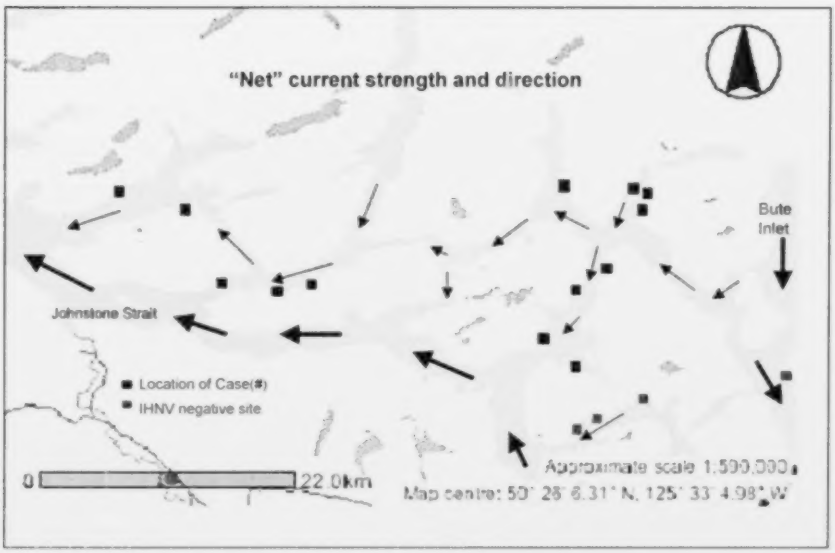


Figure 10 – Shows the direction and strength of flow of surface water in area 1
(current source: B. de Lange Boom, DFO, Ocean Sciences, Victoria BC, per.comm.)

References

Baldrock C. 2000. Epidemiology Notes. University of Tasmania. July 23-27, 2000.

Bootland L.M. and Leong J.C. 1999. Infectious Haematopoietic Necrosis Virus. In: Fish Diseases and Disorders Vol 3. Viral, Bacterial and Fungal Infections. Woo PTK and Bruno D.W. (eds). CABI Publishing, Oxon, UK. pp.57-121.

Department of Fisheries and Oceans. 1984. Fish Health Protection Regulations: manual of compliance. Fisheries Marine Services: miscellaneous special publication 31 (revised): 32p.

Emmenegger E.J., Meyers T.R., Burton T.O., and Kurath G. 2000. Genetic diversity and epidemiology of infectious hematopoietic necrosis virus in Alaska. Diseases of Aquatic Organisms, 40:163-176.

Emmenegger E.J, and Kurath, G. 2002. Genetic Characterization of Infectious Hematopoietic Necrosis Virus of Coastal Salmonid Stocks in Washington state. Journal of Aquatic Animal Health 14:25-34.

Groot C. 1996. Salmonid life histories, In Principles of Salmonid Culture ed. Pennell W., and Barton B.A. Developments in Aquaculture and Fisheries Science, vol. 29, Elsevier, Amsterdam, pp 97-230.

Hsu Y., Engelking H.M,. and Leong J.C. 1986. Occurrence of different types of infectious hematopoietic necrosis virus in fish. Applied and Environmental Microbiology 52:1353-1361.

Kahn S., Geale D.W., Kitching P.R., Bouffard A., Allard D.G., and Duncan J.R. 2002. Vaccination against foot-and-mouth disease: the implications for Canada. The Canadian Veterinary Journal. 43(5) 349-354.

Kocan R., Bradley M., Elder N., Meyers T., Bratts W., and Winton J. 1997. North American strain of viral hemorrhagic septicemia virus is highly pathogenic for laboratory-reared Pacific herring. Journal of Aquatic Animal Health. 9: 279-290.

Kent M.L., Traxler G.S., Kieser D., Richard J., Dawe S.C., Shaw R.W., Prosperi-Porta G., Ketcheson J., and Evelyn T.P.T. 1998. Survey of salmonid pathogens in ocean-caught fishes in British Columbia, Canada. Journal of Aquatic Animal Health. 10: 211-219.

McDaniel T.R, Pratt K.M., Meyers T.R., Ellison T.D., Follett J.E., Burke J.A. 1994. Alaska sockeye Salmon Culture Manual. Special Fisheries Report No.6. Alaska Department of Fisheries and Game.

Meyers T.R, Korn D., Burton T.M., Glass K., Follett J.E., Thomas J.B., Sullivan J.,Starkey N.,Short S., Lipson K. 2003. Infectious hematopoietic necrosis virus (IHNV) in Alaskan sockeye salmon culture from 1973 to 2000: annual virus prevalences and titres in broodstocks compared with juvenile losses. Journal of Aquatic Animal Health. 15:21-30.

St-Hilaire 2000. Epidemiology of infectious hematopoietic necrosis disease in net-pen reared Atlantic salmon in British Columbia, Canada. PhD thesis, University of Guelph, Guelph, Ontario.

St-Hilaire S., Ribble C.S, LaPatra S.E, Chartrand S. and Kent M.L. 2001. Infectious hematopoietic necrosis virus antibody profiles in naturally and experimentally infected Atlantic salmon *Salmo salar*. Diseases of Aquatic Organisms 46:7-14.

St-Hilaire S., Ribble C., Traxler G., Davies T., and Kent M.L. 2001. Evidence for a carrier state of infectious hematopoietic necrosis virus in chinook salmon *Oncorhynchus tshawytacha* . Disease of Aquatic Organisms 46:173-179.

St-Hilaire S., Stephen C., Kent M., Ribble C. 2001. Sentinels in the bay: using farm fish to monitor for pathogens in the environment. Journal of Marine Science 58:369-373.

Toranozo A.E. and Hetrick F.M. 1982. Comparative stability of two salmonid viruses and poliovirus in fresh, estuarine and marine waters. Journal of Fish Diseases 5:223-231.

Torgersen Y. and Hastein T. 1995. Disinfection in Aquaculture. Scientific and technical review. Office international des epizooties. 14(2) 419-434.

Traxler G.S., and Rankin J.B. 1989. An infectious hematopoietic necrosis epizootic in sockeye salmon *Oncorhyncus nerka* in Weaver Creek spawning channel, Fraser River system, B.C., Canada. Diseases of Aquatic Organisms 6:221-226.

Traxler G.S. and Richard J. 1996. First detection of infectious hematopoietic necrosis virus in marine fishes. Fish Health Section/American Fisheries Society Newsletter 24: 7.

Traxler G.S., Roome J.R., and Kent M.L. 1993. Transmission of infectious hematopoietic necrosis virus in seawater. Diseases of Aquatic Organisms 16:111-114.

Traxler G.S., Roome J.R., Lauda K.A. and LaPatra S. 1997. Appearance of infectious hematopoietic necrosis virus (IHNV) and neutralizing antibodies in sockeye salmon *Onchorynchus nerka* during their migration and maturation period. Diseases of Aquatic Organisms 28: 31-38.

Traxler G.S., Kent M.L., and Poppe T.T. 1998. Viral Diseases. In Diseases of Seawater Netpen-reared salmonid fishes. Ed Kent M.L, and Poppe T.T. Fisheries and Oceans, Canada, 138pp.

Troyer R.M., LaPatra S.E., and Kurath G. 2000. Genetic analyses reveal unusually high diversity of infectious haematopoietic necrosis virus in rainbow trout aquaculture. Journal of General Virology 81: 2823-2832.

Washburn & Gillis Associates. 1998. Guidelines for disinfection hygiene standards for aquaculture marine grow-out operations and associated services specific to infectious salmon anaemia. New Brunswick Dept. of Fisheries and Aquaculture.

Williams I.V, and Amend D.F. 1976. A natural epizootic of infectious hematopoietic necrosis in fry of sockeye salmon (*Oncorhynchus nerka*) at Chilko Lake, British Columbia. Journal of the Fisheries Research Board of Canada 33: 1564-1567.